Supporting Information

Chia et al. 10.1073/pnas.0903815106

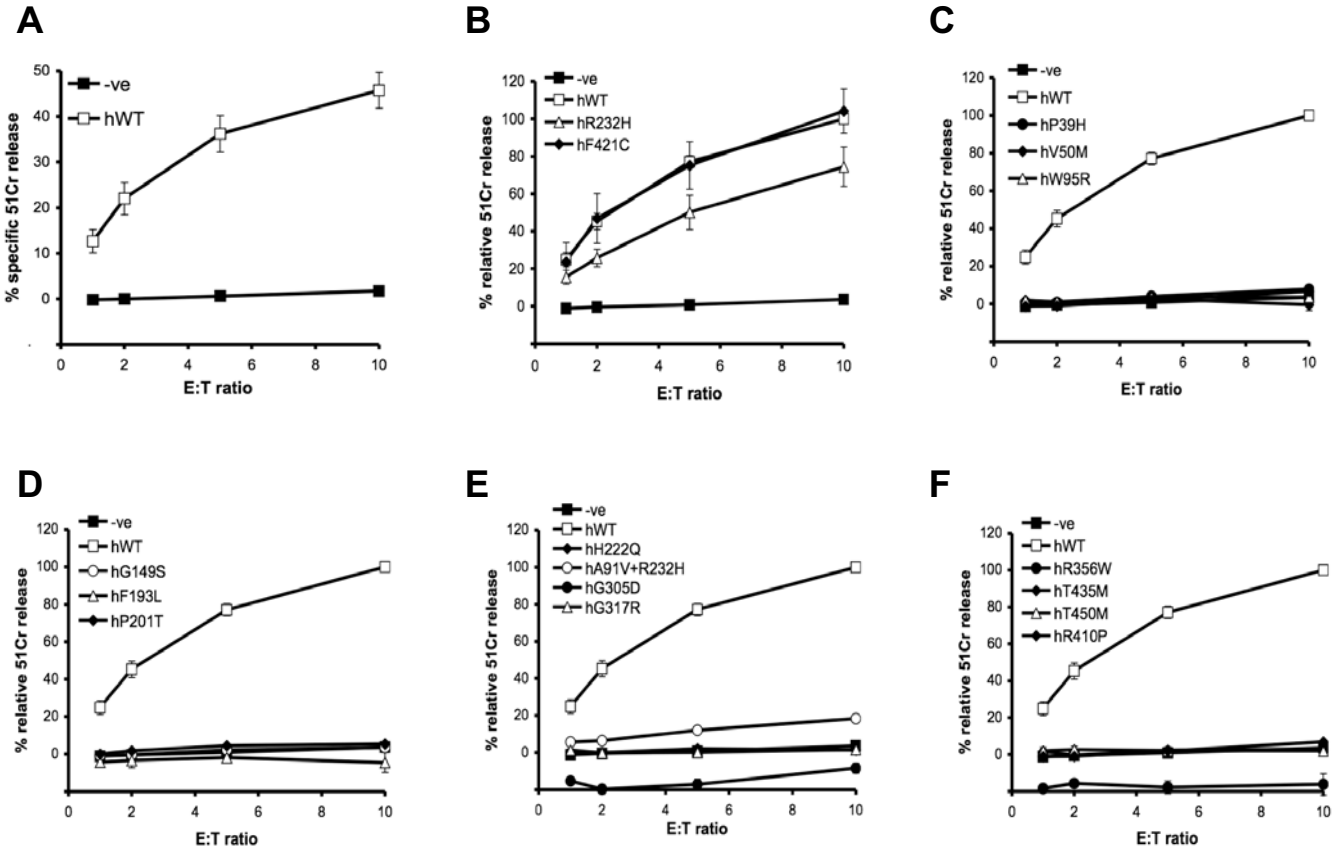

Fig. S1. All but 2 human PRF mutants identified in a cohort of patients (Table 1) fail to complement the activity *PRF1*-KO primary CTL. Primary CTLs from *PRF1*-KO mice were isolated and transfected with the human wild-type (hWT) or mutant PRF, and then sorted and tested in an antigen-restricted 4-h ^{51}Cr release assay by using SIINFEKL peptide-pulsed EL-4 target cells as described in *Materials and Methods*. (*A*) Complementation of PRF1-KO CTL (−ve) with hWT PRF. Shown is the percentage of specific ^{51}Cr release (as described in *Materials and Methods*) ± SE of 14 independent experiments. (*B*) Two mutants, hR232H and hF421C, show reduced and wild-type levels of cytotoxicity, respectively. The data shown are the mean percentage of relative ^{51}Cr release ± SE of 3–4 independent experiments for each mutant and of 14 independent experiments for hWT and control (−ve). (*C–F*) Fourteen PRF mutants show the loss of function at 37 °C. The mutants are represented in ascending order according to PRF residue number. The data shown are the mean percentage of relative ^{51}Cr release ± SE of a representative experiment for each mutant and of 14 independent experiments for hWT and control (−ve).

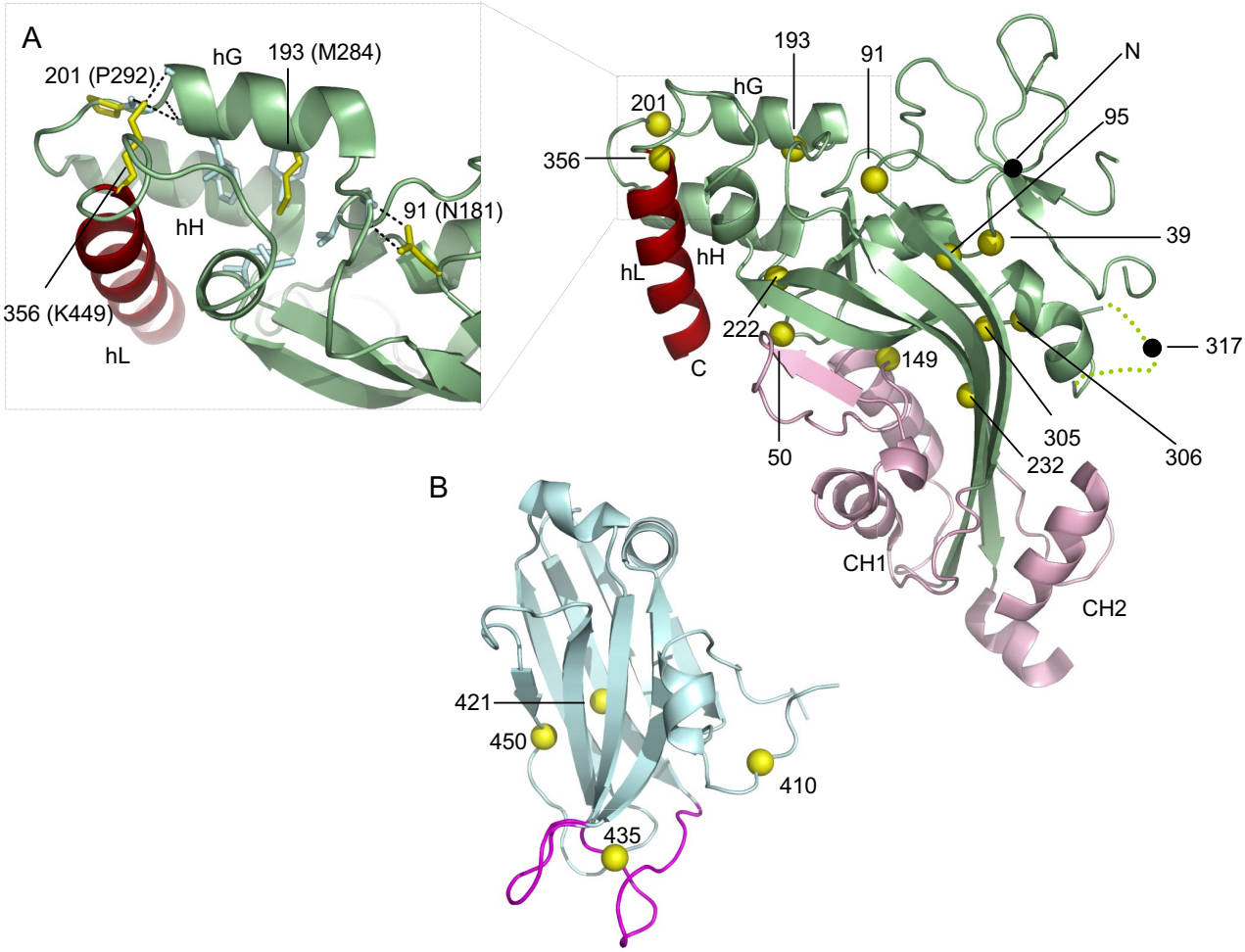

Fig. S2. Mapping PRF mutations on the predicted perforin structure. Position of the mutated perforin residues (yellow spheres) shown in Table 1 and Fig. S1 mapped onto the structure of C8α (*A*) and the archetypal C2 domain of human protein kinase Cγ (*B*). In the magnified section in *A*, the residues in C8α that are equivalent to hA91, hF193, hP201, and hR356 are shown in yellow stick and are labeled. Numbering in the panel is for human perforin with C8α numbering and amino acid identifiers shown in parentheses. M284, which is the position equivalent to hF193 in C8α, is a buried, conserved hydrophobic residue, located in the middle of the G-helix and buried in a tight hydrophobic pocket bounded by residues from the H-helix, the N-terminal region, and a short helical turn that precedes the C-terminal linker L-helix (hL). K449, which is the position in C8α equivalent to R356, caps the negatively changed dipole at the C terminus of the G-helix. The mutation of this residue to a tryptophan would be anticipated to abolish these interactions, destabilize the G-helix, and weaken the interaction between the G-helix and the L-helix. P292 (equivalent to P201) is a highly conserved residue (1, 2) located in close proximity to K449 that maps to the turn between the G- and H-helices. Finally, in C8α the position equivalent to A91, N181, interacts with the loop N-terminal to the G-helix. (*B*) Analysis of mutations in the perforin C2 domain was performed using the structure of the C2 domain of human protein kinase Cγ. The pair-wise alignment of the perforin C2 domain and the sequence of 2UZP was performed using ClustalW (3).

1. Rosado CJ, et al. (2007) A common fold mediates vertebrate defense and bacterial attack. *Science* 317:1548–1551.
2. Hadders MA, Beringer DX, Gros P (2007) Structure of C8alpha-MACPF reveals mechanism of membrane attack in complement immune defense. *Science* 317:1552–1554.
3. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

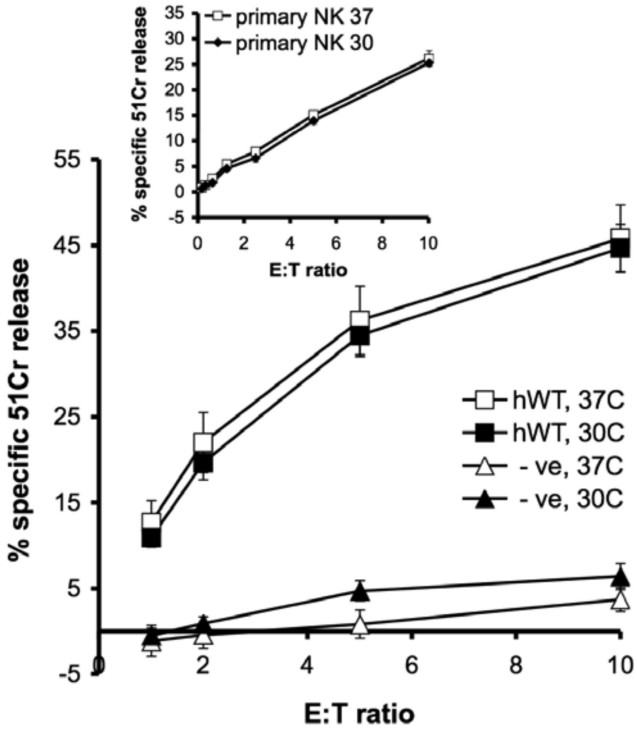

Fig. S3. The activity of hWT-transfected PRF1-KO CTL is not affected by incubation at 30 °C compared with 37 °C. Shown is the percentage of specific ^{51}Cr release of 14 independent experiments ± SE ($n = 14$). The smaller graph shows the percentage of specific ^{51}Cr release of isolated primary human NK cells incubated at 30 °C or 37 °C for 18–24 h before the cytotoxicity assay, where K562 cells were used as targets. The results are mean ± SE ($n = 3$) of a representative experiment.

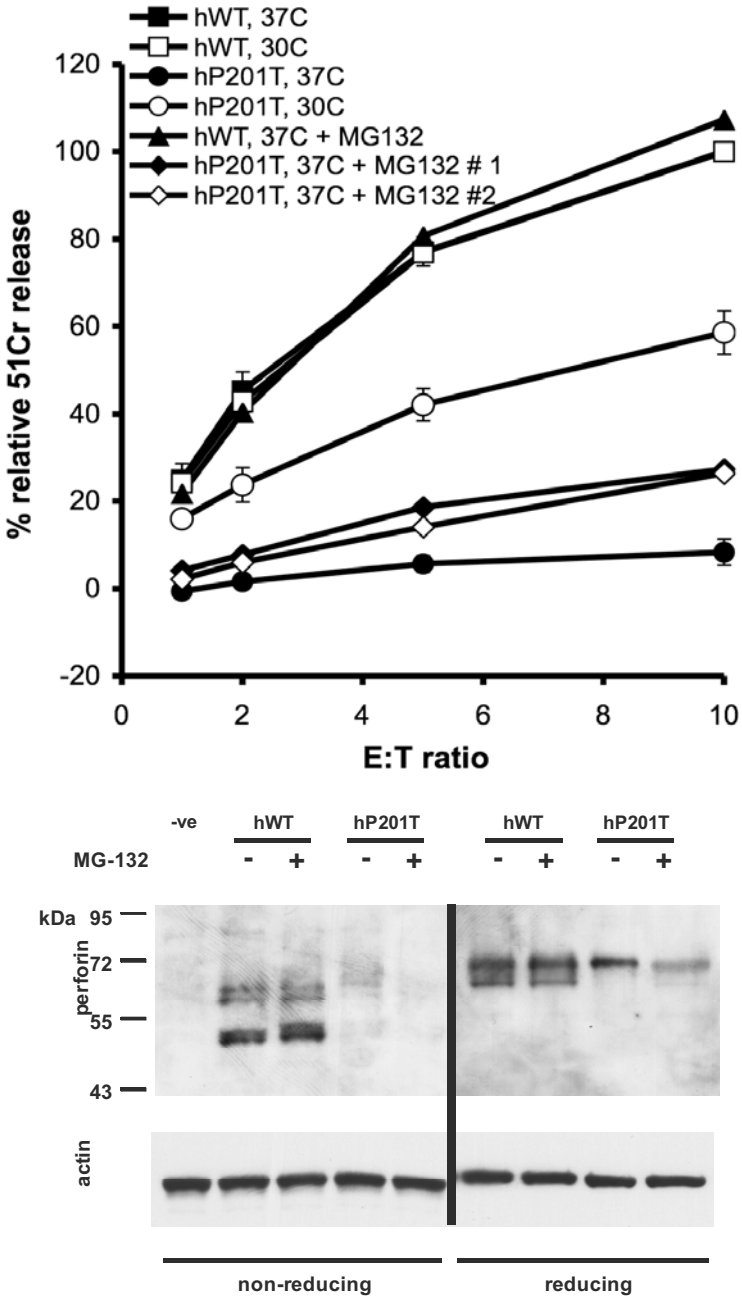

Fig. S4. Proteasome inhibitor, MG-132, fails to restore the activity of hP201T at 37 °C to the same extent as culture at 30 °C. Where indicated, PRF-transfected *PRF1*-KO CTLs were cultured for 18–24 h at 37 °C in the presence of MG-132 (0.2 μM) before cytotoxicity assay. Shown are the results of 2 independent experiments, designated #1 and #2. hP201T at 30 °C plot is from Fig. 2*A*. The proteasome inhibitor had no effect on hWT-transfected CTL activity. The Western blot on the right shows no appreciable effect of MG-132 on the expression levels of hPRF or hP201T. Lysates were prepared from transfected and sorted populations of CTL; each sample was split into 2 aliquots and subjected either to reducing or nonreducing SDS/PAGE. hP201T was undetectable under nonreducing conditions, but was comparable to hWT expression level on the reducing gel.